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STUDIES OF THE DIFFUSION OF CHROMIUM COMPOUNDS THROUGH SKIN*

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In a previous report (1) on patch test reactions of chromate-sensitive subjects to trivalent chromium compounds, we postulated that two factors must be taken into account in order to interpret the results: the penetrability of the compound through the epidermal barrier, and the concentrations of the test solutions.

Skog and Wahlberg (2, 3) showed through "disappearance measurements" of radioactive compounds from the cutaneous surface that there was little difference in the diffusion of Na_2CrO_4 and $CrCl_3$ at concentrations below 0.24 M. However, the hexavalent species became more diffusible than the trivalent form as the concentration increased above 0.24 M, and plateaus were reached slightly above this value. By a similar technic, Schwarz and Spier (4) observed that there is little difference in the absorption of $K_2Cr_2O_7$ and $CrCl_3$ and concluded that differences in absorption cannot explain the differences in sensitization potency. In a preliminary study (5), chemical determinations on the diffusion of chromium compounds through skin revealed marked differences in the penetrating abilities of various trivalent chromium compounds depending on the nature of the acid radical. Diffusion of $chromium_{(III)}$ in the form of sulfate was negligible; it was slight as nitrate, while as chloride it was comparable to $Cr_{(VI)}$.

The significance of these penetration studies becomes apparent when we consider the contradictory results reported when various chromium salts are used in patch testing. Fregert and Rorsman (6) were able to elicit positive patch test reactions in 11 of 17 chromate-sensitive subjects with 0.5 M $CrCl_3$ solutions; at lower concentrations (0.07 M $CrCl_3$) only 4 of 22 chromate-sensitive subjects showed positive patch tests. On the other hand, Cohen (7) reported negative patch test reactions to chromic chloride (approximately 0.01 M) in 44 chromate-sensitive subjects. In our studies (1, 5) we were rarely able to produce positive patch test reactions with 0.01 to 0.2 M solutions of $CrCl_3$, $Cr_2(SO_4)_3$ and $Cr(NO_3)_3$. We have hypothesized that this was due to the slower penetration of the lower concentrations of these compounds, especially the sulfate and nitrate, in comparison to $K_2Cr_2O_7$. To test this hypothesis the following studies were carried out.

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EXPERIMENTAL

I. *Diffusion Studies*

Epidermis removed from autopsy skin was sealed between the upper and lower parts of the diffusion apparatus (Fig. 1) with silicon grease. The lower part of the apparatus was filled with buffered saline; a measured volume of the tracer-carrier chromium solution was diluted with the desired buffer and introduced into the upper part of the apparatus. The diffusion apparatus was supported in a water bath at 37° C. The contents

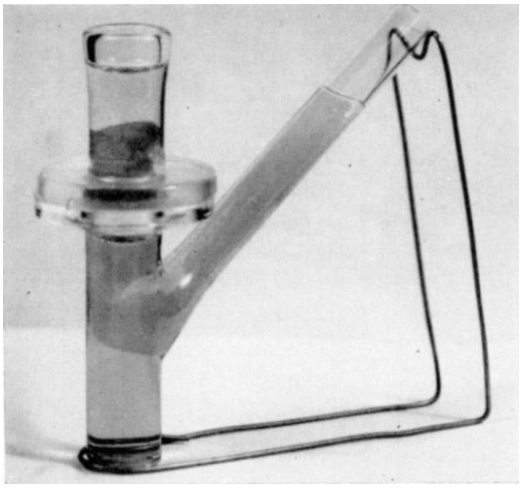


FIG. 1. Diffusion apparatus. Autopsy skin is maintained in place between upper and lower parts.

of the lower part of each apparatus were stirred by means of small rotating magnetic stirrers. One ml aliquots were withdrawn hourly from the lower reservoir through the side arm and assayed in a well-type scintillation counter. Chromium concentrations were calculated from a calibration curve made by radio assay of dilutions from the original tracer-carrier solutions.

II. *Ionic Charge Studies*

Dowex ion exchange resins were converted to the desired ionic form by treatment with either potassium chloride, sulfate or nitrate solutions buffered with phosphate. Measured aliquots of the tracer-carrier chromium solutions were mixed with the desired buffer-salt solution and allowed to stand 24 hours to insure equilibrium. Half ml aliquots were introduced to the prepared ion exchange columns and eluted with the corresponding buffer solutions.

Fractions were collected every five ml and assayed for radioactivity. Recovery was calculated in relation to the activity of 0.5 ml of a 1:10 dilution of the original mixture.

Results and Discussion

The effect of chromate concentration of the diffusion of chromium from 0.2 M KCl at pH 7 is presented in Figure 2. These data

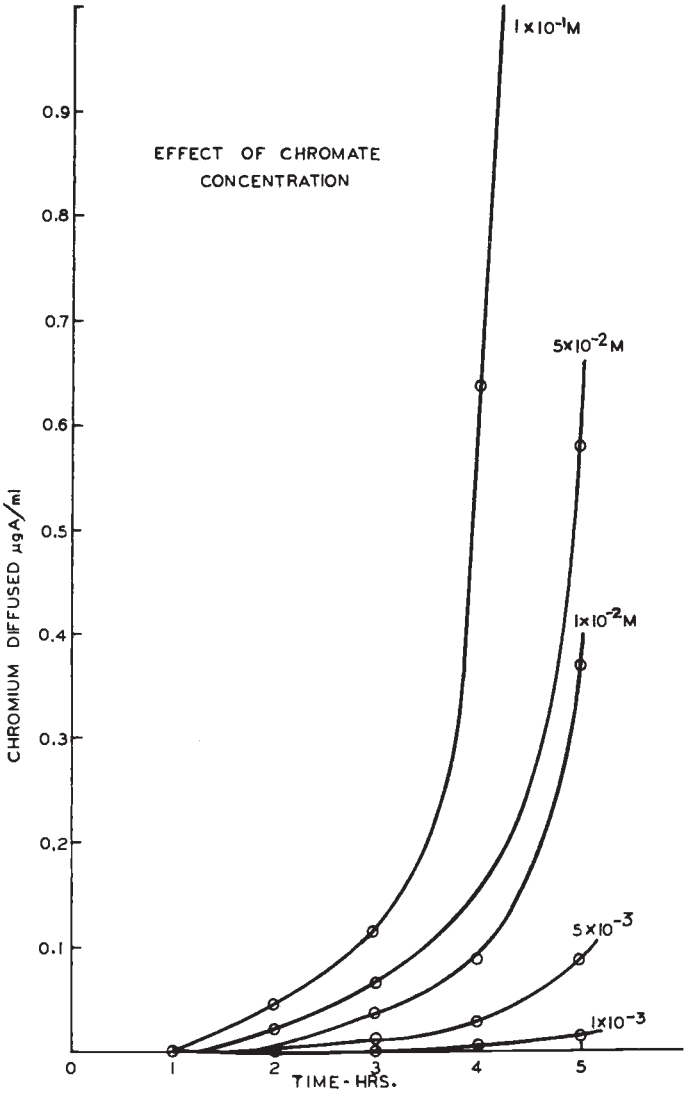
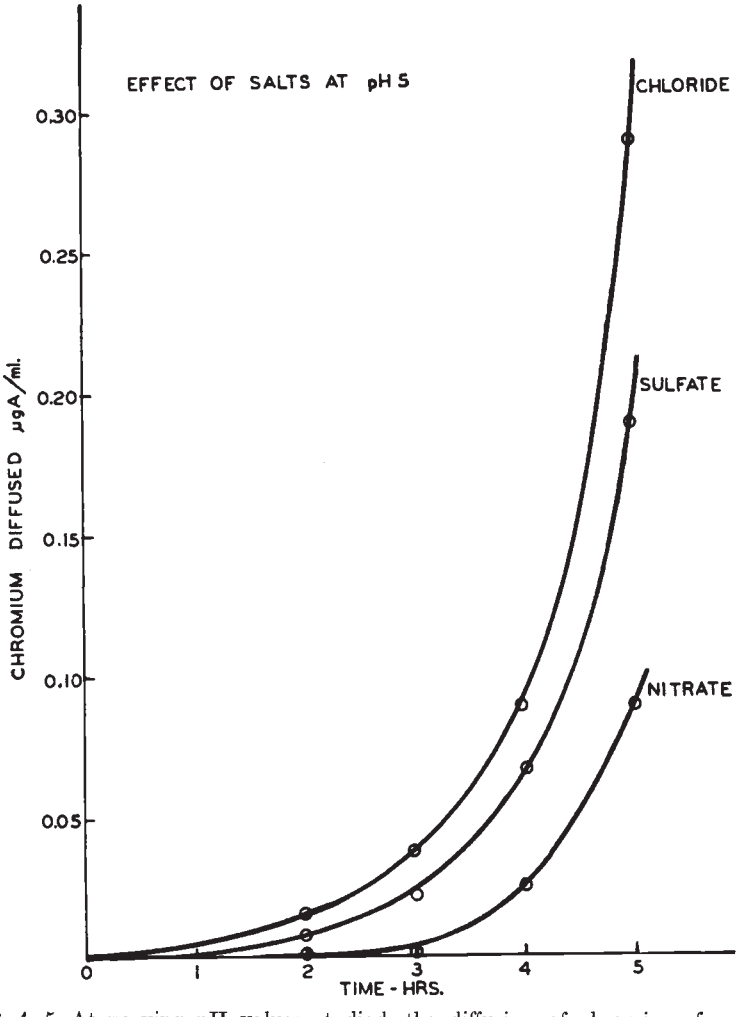


Fig. 2. The diffusion of chromium increases with increasing chromium concentration



FIGS. 3, 4, 5. At varying pH values studied, the diffusion of chromium from chloride solutions was always greater than from sulfate or nitrate solutions. At pH 7 there is less diffusion than at pH 5 or pH 9.

show that the diffusion of chromium increases with increasing chromate concentration over a 1×10^{-3} to 1×10^{-1} M range. This concentration range compares with that found in industry (wood preservation, 1×10^{-1} M; antifreezes, 1×10^{-3} M; corrosion inhibitors, 5×10^{-3} M; tanning liquor, 2×10^{-1} M). A similar observation was made on the diffusion of chromium from solutions of the trivalent species 0.2 M in KCl and buffered at pH 7. In this case, however, the amounts of chromium found in the lower part of the appartus were 50 to 75% less than those found in diffusion from similar solutions of hexavalent chromium. These observations are in accord with simple diffusion theory; the lower diffusion of trivalent chromium can be attributed to its lesser solubility.

The data in Figures 3, 4 and 5 demonstrated the effect of pH and acid radicals (anions) on the diffusion of chromium from solutions of the trivalent form. At the pH values studied, diffusion from chloride solutions was always greater than that from sulfate or nitrate solutions; diffusion from all solutions appeared to be lower at pH 7 than at pH 5 or 9. The latter observation is attributed to minimal solubility of trivalent chromium compounds near neutrality. The variations in diffusion from chloride, sulfate

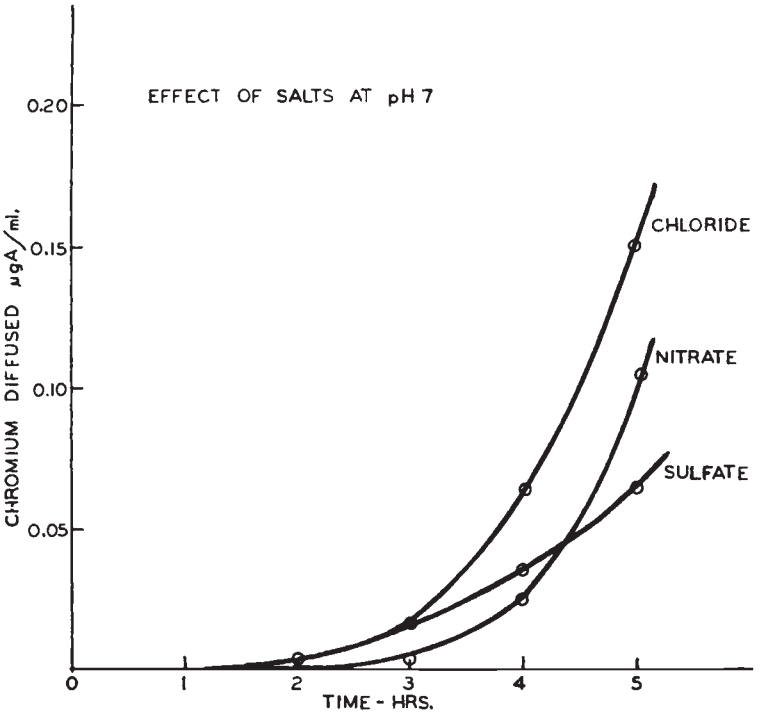


FIG. 4

or nitrate solution indicate that the acid radical may be involved in the formation of a "diffusible species".

We were unable to produce allergic reactions to chromium nitrate which, depending upon the concentration and pH, is 3 to 30 times less diffusible than the hexavalent species. The diffusion from sulfate solutions is between that from chloride and nitrate solutions. However, chromic chloride, which is only 2 to 4 times less diffusible, has been reported to cause allergic reactions in chromate sensitive subjects (6). In this respect Mali and coworkers (8) have found that in chromate-sensitive subjects at least 20 times more trivalent chromium must be used to produce the same epicutaneous results as hexavalent chromium.

The differences in diffusion of trivalent and hexavalent chromium compounds can be correlated with their ability to elicit allergic reactions, by assuming that the chromium must diffuse through the epidermis before it can form a complete antigen, and that only negatively charged ions can cross the barrier. The formation of such negatively charged ions can take place by the combination of the positively charged trivalent chromic ion with acid radicals; *i.e.*,

$$[Cr(H_2O)_6]^{+3} + 4Cl^- \rightarrow [CrCl_4]^- + 6H_2O$$

The combining properties of nitrate are less than those of chloride or sulfate, therefore, fewer negatively charged complexes are formed in nitrate solutions and diffusion is lowest from nitrate solutions. The hexavalent chromium ion is entirely negatively charged and diffusion is thus greatest.

In our ion exchange studies we assumed that the cation exchange resins would exchange only the positively charged ionic species from the "trivalent chromium buffer-salt" solutions, and that the column effluent would contain the negatively charged complexes. The elution curves in Figure 6 shows the presence of at least two anionic or neutral, or both types of chromium complexes. Of the chromium used in these studies, 40.3 and 41.9% was present in the form of these complexes in the chloride and sulfate solutions respectively. Only 11.9% of the chromium in the nitrate solutions passed through the cation

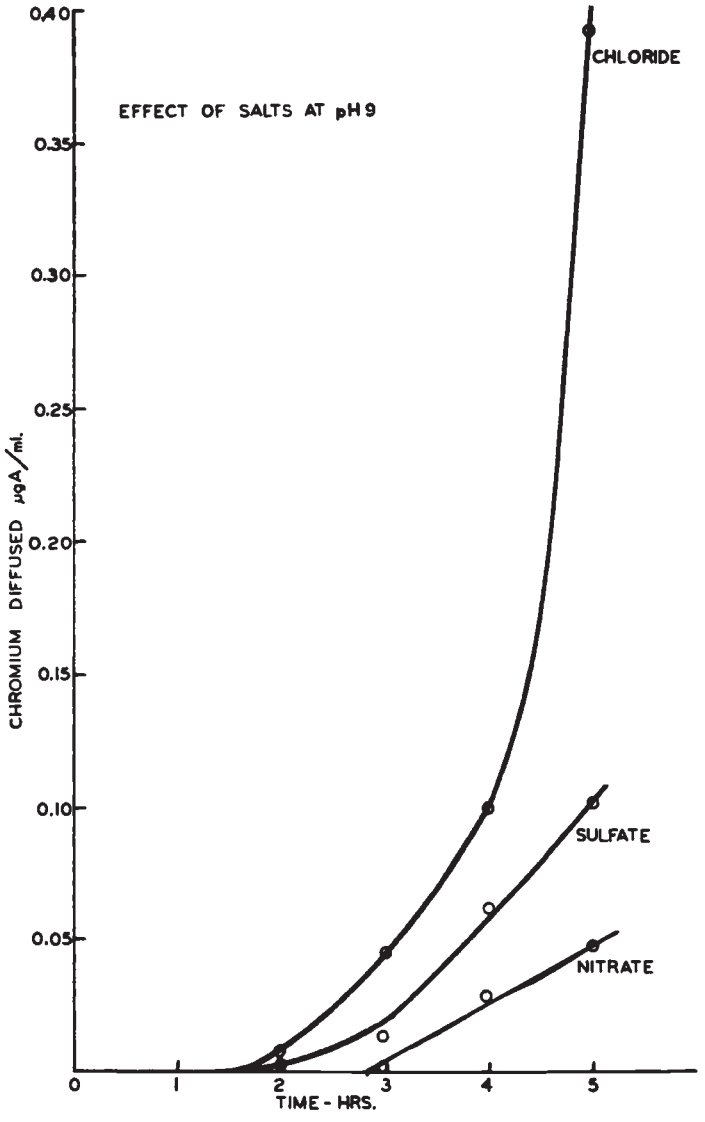


FIG. 5

exchange column, indicating fewer anionic species. It is impossible to define these complexes at present; however, there appears to be one species common to all three systems.

The elution curves presented in Figure 7 were obtained with an anion exchange resin. In this case, negatively charged ions are exchanged. These curves indicate that similar cationic or neutral chromium species are present in all three systems. Of the total chromium used, 71.0, 53.5 and 87.9% was recovered from the anion exchange columns used to investigate the chloride, sulfate and nitrate systems respectively.

From the above data and from previous work (9), we should like to propose the following partial sequence of events for allergic reactions to chromium. A chromium compound is applied to the skin of a chromium-sensitive subject. If the compound is hexavalent, it rapidly diffuses into the skin and undergoes chemical reduction to some triva-

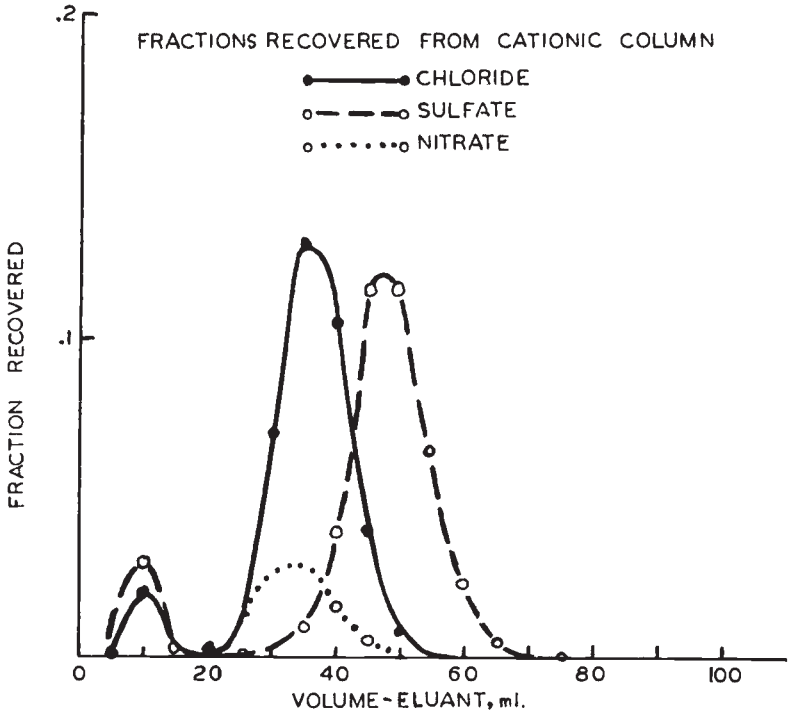


FIG. 6. Cation exchange resins theoretically exchange only the positively charged ionic complexes. These elution curves show that the nitrate solution has fewer anionic, or negatively charged, species than the chromium chloride, or the chromium sulfate solution.

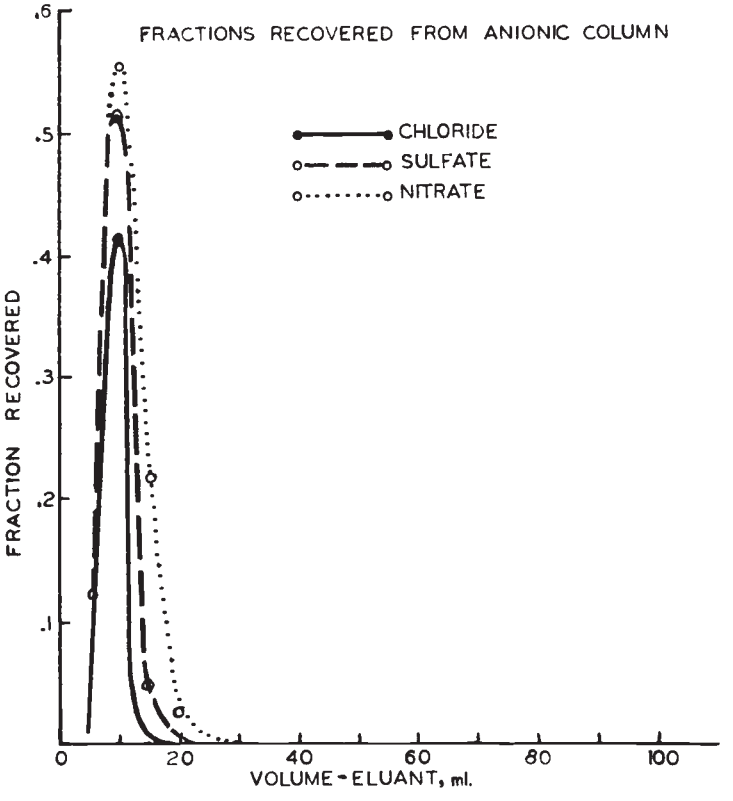


FIG. 7. Anion exchange resins exchange only negatively charged ions. Here we see that the nitrate solution has more cations than either the chloride or sulfate.

lent form. This trivalent form unites *in vivo* with an appropriate protein carrier to form the complete antigen. If the chromium compound applied to the skin is trivalent, it diffuses more slowly. However, in the presence of large amounts of chloride or at high concentrations, considerable amounts of chromium can diffuse through the skin at a rate approaching that of the hexavalent species. The trivalent chromium then can combine with a protein carrier to form a complete antigen as in the case of the hexavalent species.

It remains to be established whether the reacting carriers are the same, whether there is a common determinant group in the carrier or whether a cross re-activity may exist between two different carriers.

In previous clinical studies (5), it has been shown that the principal mode of interaction of hexavalent chromium is not one of oxidation as far as antigen formation is concerned. Additional laboratory studies are now in progress to further substantiate that the oxidized proteins is not the antigen and that the chromium ion is essential in the formation of a metal-protein complex which acts as the antigen. However, it is possible that the redox reaction with $Cr_{(VI)}$ provides a protein protein carrier with stronger affinity for binding, or that the trivalent chromium as might develop from the interaction, is predisposed to form a hapten-protein complex with a linkage of greater relevancy. These possibilities have not been examined yet in sufficient detail to decide whether or not they are valid.

SUMMARY

The penetration of chromium compounds through isolated human epidermis as a function of the ionic strength, pH and ionic makeup of the systems is reported.

The correlation of penetration through the epidermis and the concentrations of the test solutions with the capacity of trivalent compounds to act as elicitors in chromate-sensitive subjects is discussed.

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